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PANCREATIC ACINAR CELL FUNCTION: MEASUREMENT OF INTRACELLULAR IONS AND pH AND THEIR RELATION TO SECRETION

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SUMMARY

1. Isolated mouse pancreatic acini were used to investigate the effect of secretagogues on acinar cellular electrolytes and cell pH. The effect of changes in the acid–base status of the incubation medium on acinar cellular electrolytes, cell pH and amylase release were also studied.

2. Carbachol at concentrations of 10^{-6} or 10^{-5} M was without any effect on the intracellular concentrations of total Na^+, Na^+ exchangeable with $^{22}Na^+$, K^+ and Cl^-, and did not influence cell pH as determined by the DMO method.

3. Changes in pH_e achieved by varying the HCO_3^- concentration at constant CO_2, varying the CO_2 concentration at constant HCO_3^- or by titration of a HCO_3^-/CO_2 free HEPES buffered medium did not influence intracellular electrolyte values.

4. pH_i changed linearly with pH_e by about 1·2 units/pH_e unit change over the pH_e range of 7·7–6·5. pH_i, however, did not change in response to a change in the CO_2 tension when the HCO_3^- concentration was adjusted to keep pH_e at 7·4.

5. Lowering pH_e below 7·1 inhibited carbachol and CCK_8-stimulated amylase release. By contrast a decrease of pH_e to 6·8 was without significant effect on basal and secretagogue increased $^{45}Ca^{2+}$ efflux from pancreatic acini.

6. In conclusion the pH sensitivity of amylase release by acinar cells is probably related to changes in pH_i. Since Ca^{2+} mobilization is not affected, the pH sensitive step is probably in the mechanism by which Ca^{2+} activates the release of zymogen granules contents by exocytosis.

INTRODUCTION

A prominent feature of pancreatic acinar cell stimulus–secretion coupling is the electrophysiological change in membrane potential and resistance, which has been interpreted as being due to underlying changes in plasma membrane permeability to specific ions (Nishiyama & Petersen, 1975; Iwatsuki & Petersen, 1977). The importance of these changes in the control of digestive enzyme secretion however is still not well established (Williams, 1981). Rather the central step in the control of secretion is believed to be an increase in cytoplasmic free Ca^{2+}, which is released from intracellular stores (Chandler, 1978; Case, 1978; Williams, 1980). Since agents which

stimulate release of digestive enzymes from pancreatic acini also increase the sodium permeability of the plasma membrane (Nishiyama & Petersen, 1975; Iwatsuki & Petersen, 1977) and increase the influx of $^{22}Na^+$ into the cell (Case, Clausen & Scott-Wilson, 1978; Bobinski & Kelly, 1979; Putney, Landis & Van de Walle, 1980), it has been suggested that Na^+ influx into acinar cells might be the mechanism which leads to the release of Ca^{2+} from intracellular stores in response to stimulation (Case & Clausen, 1973; Williams, 1975). This possibility has gained support from the observation that in other cell types an increase in sodium can bring about release of Ca^{2+} from mitochondria (Crompton, Künzi & Carafoli, 1977; Crompton, Moser, Ludi & Carafoli, 1978). It is also known that changes in intracellular pH can affect uptake or release of intracellular Ca^{2+}, which is important in stimulus–contraction coupling (Carvalho & Leo, 1967; Williamson, Safer, Rich, Shaffer & Kobayashi, 1975) and stimulus–secretion coupling (Malaisse, Herchuelz & Sener, 1980). To gain further insight into regulatory mechanisms involved in the release of digestive enzymes from pancreatic acini we investigated the effects of secretagogues on the intracellular concentrations of sodium, potassium and chloride and on cell pH as well as the effect of changes in extracellular pH on amylase release, cellular electrolytes, and cell pH.

METHODS

Tissue preparation and incubation

All studies were performed using white male Swiss mice, weighing 20–25 g which were fasted for 16–18 h before use and killed by decapitation. Isolated pancreatic acini were prepared by enzymatic digestion as previously described (Williams, Korc & Dormer, 1978). Briefly, the procedure includes injection of digesting medium, containing the enzymes hyaluronidase, purified collagenase, and chymotrypsin into the interstitium of pancreatic tissue. After incubation at 37 °C for 50 min with shaking (120 cycles/min), acini were dissociated by shearing through a constrictive pipette orifice and purified by filtration and centrifugation (4 min at 50 *g*) through medium containing 4 % bovine serum albumin. Unless mentioned otherwise, the isolated acini were preincubated for 60 min in physiological media (see below), then centrifuged, rinsed and resuspended in fresh media of specified composition and incubated for 30 min, during which time period specified isotopes were added. The average acinar density was about 1·3 mg acinar protein/ml medium.

The standard incubation medium was a modified Krebs-Henseleit bicarbonate buffer (KHB) of the following composition: glucose, 5·5 mM; NaCl, 118 mM; KCl, 4·7 mM; $NaHCO_3$, 25 mM; Na_2HPO_4, 1 mM; $MgCl_2$, 1·13 mM; $CaCl_2$, 1·28 mM. The medium was supplemented with soybean trypsin inhibitor, 0·1 mg/ml, bovine serum albumin 2 mg/ml and Minimal Eagles Medium amino acid supplement neutralized with NaOH (Williams *et al.* 1978). The medium was equilibrated with 95 % O_2/5 % CO_2 and the temperature was kept constant at 37 °C. In order to change the extracellular pH (pH_e) the HCO_3^- concentration was either raised to 50 mM (pH_e 7·7) or reduced to 12·5 mM (pH_e 7·1), 6·3 mM (pH_e 6·8) or 3·1 mM (pH_e 6·5). For other experiments the medium was equilibrated with 80 % O_2/20 % CO_2 while the HCO_3^- concentration was either kept constant (pH_e 6·8) or raised to 100 mM (pH_e 7·4). In all cases pH_e was, if necessary, adjusted to the defined value at 37 °C. In most experiments the stability of pH_e during the experiment was monitored by incubation of two flasks without acini from which pH_e was measured at the end of the incubation time with a regular pH electrode. Alternatively, the pH of small samples of the cell suspension was determined under anaerobic conditions by means of a Radiometer blood gas electrode (PHM 73). In some cases CO_2/HCO_3^- was omitted and the medium was buffered with 10 mM *N*-2-hydroxyethylpiperazinyl-*N'*-2-ethanesulphonic acid (HEPES), with pH_e adjusted with NaOH at 37 °C to values varying between 6·5 and 7·7. HEPES-buffered Ringer media (HR) were gassed with 100 % O_2. The osmolality of all solutions was kept constant by adjustment of NaCl.

Determination of amylase and protein

Measurement of amylase released into the medium during the 30 min incubation period was carried out as previously described (Williams *et al.* 1978). Release is expressed as a percentage of the total acinar amylase content present at the beginning of the incubation time. Amylase activity was assayed by the method of Jung (1980) utilizing procion yellow starch as a substrate. The starch substrate was prepared by coupling procion yellow (MX-8G, Polyscience, Warrington, PA) to starch. Protein was assayed by the method of Lowry, Rosebrough, Farr & Randall (1951) using bovine serum albumin as standard. CCK_8 was a gift from Dr M. Ondetti, Squibb Institute for Medical Research, Princeton, NJ, U.S.A.

Determination of electrolytes

For determination of total and extracellular H_2O and uptake of Na, cells were incubated for 5 min with a combination of [^{3}H]H_2O (2·5 μCi/ml) and [^{14}C]inulin (0·5 μCi/ml) or for 30 min with ^{22}Na (1 μCi/ml) with [^{3}H]inulin (2 μCi/ml) added 5 min before the end of the incubation time, to avoid the small but significant uptake of inulin into the cells, which was observed at longer incubation times. One ml aliquots of acinar suspension were then centrifuged for 1 min at 10,000 rpm in an Eppendorf microcentrifuge, the supernatant was removed, the pellet dispersed in 1 ml H_2O, and the radioactivity in the pellet and the supernatant was measured by liquid scintillation counting in 10 ml of a Triton: toluene (2:1) butyl-PBD (4 g/l) cocktail. In other experiments, cells were incubated with [^{14}C]5,5-dimethyl-2,4-oxazolidinedione (DMO, 0·5 μCi/ml) and [^{3}H]inulin (2 μCi/ml). After centrifugation, total pellet water was determined by weighing before and after drying at 80 °C for 24 h. Samples were then extracted with 0·1 N-HNO_3 for 36–48 h at room temperature (Williams, 1975). The extracted Na^+ and K^+ content was analysed by flame photometry using an Instrumentation Laboratories machine (type 343), utilizing Li^+ as an internal standard. Chloride was determined by electrometric titration, using a Buchler Cotlove chloridometer. The [^{3}H]inulin space was used to calculate the intracellular concentrations from the total electrolyte values. The electrolyte and ^{22}Na data are expressed as mmol/l cell H_2O. The cell pH (pH_i) was calculated from the cellular and extracellular distribution of [^{14}C]DMO, with the use of a pK value of 6·13 (Waddell & Butler, 1959). All isotopes were obtained from New England Nuclear, Boston, MA, U.S.A.

Determination of $^{45}Ca^{2+}$ efflux

Isolated pancreatic acini were allowed to recover for 30 min and then preincubated for 1 h in KHB, containing $^{45}Ca^{2+}$ (2 μCi/ml; total Ca^{2+} = 1·28 mM). The cells were then centrifuged for 2 min at 50 *g* and the pellet washed and resuspended in isotope-free medium of specified pH but containing the same amount of non-radioactive Ca. Immediately following resuspension an aliquot was taken to measure total acinar $^{45}Ca^{2+}$ content. Then at different time points (1–20 min), 1 ml aliquots were centrifuged and the $^{45}Ca^{2+}$ in the supernatant was determined by liquid scintillation counting. The amount of radioactivity in the medium was subtracted from total acinar $^{45}Ca^{2+}$ and the $^{45}Ca^{2+}$ content of the acini at each time point was expressed as percentage of $^{45}Ca^{2+}$ present at the beginning of the washout period.

RESULTS

Effect of carbachol on cellular electrolytes and cell pH

The intracellular electrolyte content and cell pH (pH_i) of mouse isolated pancreatic acini were determined after 30 min incubation in the absence (control) and in the presence of carbachol with the results shown in Table 1. In unstimulated cells, the average $[K^+]_i$ was 141 ± 5·1 mM, $[Na^+]_i$ was 52·5 ± 3·5 mM and $[Cl^-]_i$ was 53·7 ± 5·0 mM. Carbachol at a concentration of 10^{-6} M, which induces maximum stimulation of amylase release, or 10^{-5} M, which induces maximum stimulation of $^{45}Ca^{2+}$ efflux from isolated pancreatic acini (Williams *et al.* 1978) was without significant effect on any of these intracellular ion concentrations. By use of [^{3}H]H_2O and [^{14}C]inulin, cellular water was determined to be 3·9 ± 0·1 μl/mg protein (n = 10) and was also not affected by the addition of carbachol.

Average intracellular pH (pH_i) was calculated from the distribution of [^{14}C]DMO between intra- and extracellular water according to the method developed by Waddell & Butler (1959). The value of extracellular pH (pH_e) was determined on small aliquots of the cell suspension, taken anaerobically at the end of the incubation. The average pH_e under control conditions was $7{\cdot}42 \pm 0{\cdot}01$, while the pH_e at stimulatory carbachol concentrations was $7{\cdot}39 \pm 0{\cdot}01$ and therefore slightly more acid ($P < 0{\cdot}05$). The calculated pH_i for control acini was $7{\cdot}00 \pm 0{\cdot}07$; no change could be observed in carbachol treated cells.

TABLE 1. Effect of carbachol on cellular electrolytes and pH_i of isolated pancreatic acini

Carbachol	$[Na^+]_i$ (mM)	$[K^+]_i$ (mM)	$[Cl^-]_i$ (mM)	pH_e	pH_i
None	$52{\cdot}5 \pm 3{\cdot}5$ (9)	$141 \pm 5{\cdot}1$ (9)	$53{\cdot}7 \pm 5{\cdot}0$ (8)	$7{\cdot}42 \pm 0{\cdot}01$ (5)	$7{\cdot}00 \pm 0{\cdot}07$ (6)
10^{-6} M	$54{\cdot}5 \pm 3{\cdot}9$ (9)	$144 \pm 4{\cdot}4$ (9)	$52{\cdot}0 \pm 5{\cdot}0$ (8)	$7{\cdot}39 \pm 0{\cdot}01$ (4)	$6{\cdot}99 \pm 0{\cdot}09$ (6)
10^{-5} M	$56{\cdot}9 \pm 3{\cdot}5$ (8)	$140 \pm 4{\cdot}1$ (8)	$56{\cdot}2 \pm 5{\cdot}1$ (7)	$7{\cdot}39 \pm 0{\cdot}01$ (3)	$7{\cdot}00 \pm 0{\cdot}06$ (5)

All values are the mean ± S.E. of the number of experiments indicated in parenthesis.

TABLE 2. Effect of carbachol on total and exchangeable acinar sodium

Carbachol	Total $[Na^+]$	Exchangeable [$^{22}Na^+$]	% exchangeable
None	$51{\cdot}0 \pm 6{\cdot}0$	$48{\cdot}5 \pm 3{\cdot}0$	$98{\cdot}9 \pm 5{\cdot}3$
10^{-6} M	$51{\cdot}2 \pm 7{\cdot}8$	$49{\cdot}3 \pm 4{\cdot}2$	$98{\cdot}9 \pm 8{\cdot}6$
10^{-5} M	$54{\cdot}4 \pm 7{\cdot}0$	$54{\cdot}8 \pm 2{\cdot}8$	$102{\cdot}9 \pm 8{\cdot}8$

All values are the mean ± S.E. of three experiments.

Although carbachol did not affect the total acinar sodium content it is possible that some intracellular Na^+ may be bound and that secretagogues influence the concentration of a smaller exchangeable pool of Na^+. We therefore evaluated the exchange of acinar Na^+ with $^{22}Na^+$ in the presence and absence of carbachol. Acini were incubated for 30 min with $^{22}Na^+$, a time period over which the $^{22}Na^+$ uptake into isolated acinar cells had reached a steady state (Putney *et al.* 1980). The results shown in Table 2 indicate that all cellular sodium was exchangeable with $^{22}Na^+$ and the $^{22}Na^+$ content was also not affected by carbachol.

Effect of pH_e on amylase secretion, cellular electrolytes and pH_i

Effect of varying HCO_3^- concentration. The effect of altering the pH_e by changing the HCO_3^- concentration of KHB equilibrated with 95% O_2/5% CO_2 on basal and stimulated amylase release is shown in Fig. 1. Basal amylase secretion was not altered by changes in the pH of the incubation medium, while amylase secretion stimulated by carbachol (10^{-6} M) or cholecystokinin octapeptide (CCK_8) (7×10^{-11} M) was significantly reduced, when pH_e was lowered to 6·8. In contrast to this change in amylase release, varying pH_e at a constant CO_2 did not influence $[K^+]_i$ and $[Na^+]_i$ (Fig. 2). pH_i, however, decreased when pH_e was lowered and increased when pH_e was raised. The experimental points suggest a linear relationship between pH_i and pH_e over pH_e range tested. The slope of this relationship indicates a change of 1·2–1·3 pH_i units for a unit change in pH_e.

Effect of varying CO_2. To investigate further whether the inhibition of secretagogue-

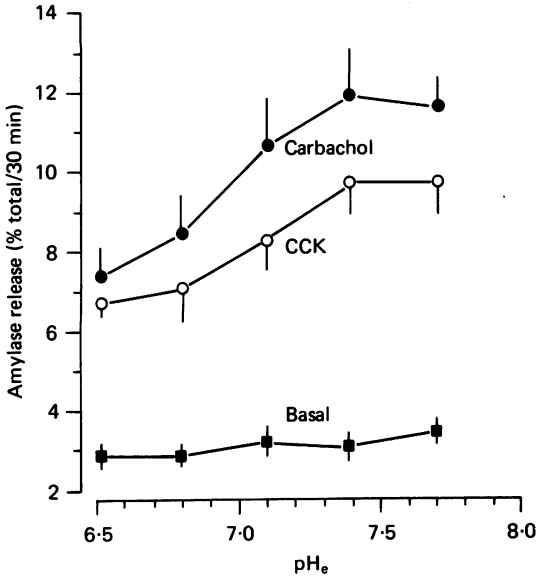

Fig. 1. Effect of varying extracellular pH (pH_e) on amylase release from isolated mouse pancreatic acini incubated in KHB in the absence (basal) and in the presence of 10^{-6} M-carbachol (●) and 7×10^{-11} M-CCK_8 (○). Amylase release is expressed as a percent of total acinar amylase content. Each point is the mean ± S.E. of six experiments in each of which duplicate determinations were carried out at every value of pH_e. pH_e was adjusted by varying the HCO_3^- concentration and equilibrating all solutions with 95% O_2/5% CO_2.

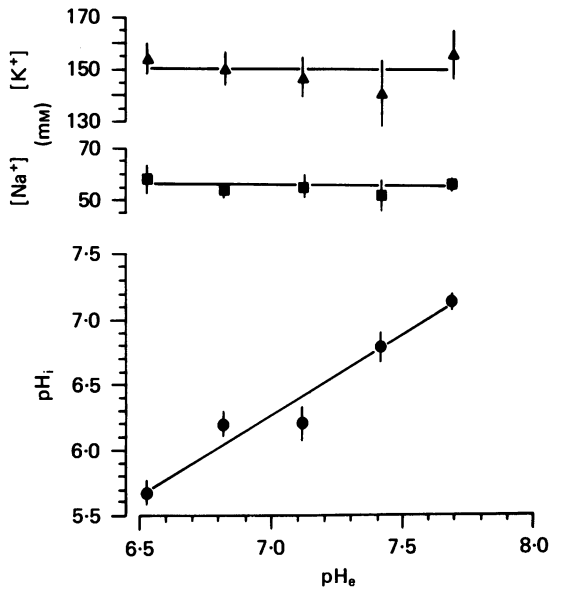

Fig. 2. Effect of varying extracellular pH (pH_e) on the cellular content of K^+ and Na^+ and on the intracellular pH (pH_i). Representative experiment of two. Each point is the mean ± S.D. of six determinations. Acini were incubated for 30 min in KHB in which pH_e was adjusted by varying the HCO_3^- concentration and equilibrating with 95% O_2/5% CO_2.

induced amylase secretion can be directly related to changes in pH_e or to changes in pH_i, the CO_2 concentration of the KHB-medium was increased to 20%. This is known to lower pH_i in other tissues (Caldwell, 1958; Thomas, 1974; Boron & DeWeer, 1976), even if the HCO_3^- concentration is raised to keep pH_e at 7·4. The effects of changes in the acid-base status on cellular electrolytes, pH_i and amylase secretion are enumerated in Table 3. Equilibration of KHB with 20% CO_2 did not affect pH_i, $[K^+]_i$ or $[Na^+]_i$, nor basal and stimulated amylase release as long as pH_e was kept

TABLE 3. Effect of varying extracellular acid-base status on intracellular electrolytes and amylase release

	Intracellular electrolytes				Amylase release (% total/30 min)			
Extracellular acid-base status	pH_i	$[K^+]_i$ (mM)	$[Na^+]_i$ (mM)	n	Control	Carbachol (10^{-6} M)	CCK_8 (7×10^{-11} M)	n
5% CO_2	6·89	164	59·5	3	2·9	11·0	7·7	4
pH_e 7·4	±0·02	±8·9	±5·1		±0·3	±1·1	±1·2	
20% CO_2	6·89	162	58·2	3	2·5	9·4	7·3	4
pH_e 7·4	±0·06	±5·6	±4·5		±0·5	±1·1	±0·7	
20% CO_2	6·35	159	58·9	3	2·0	6·8	5·2	4
pH_e 6·8	±0·16	±11·5	±5·6		±0·3	±0·9	±0·7	

All values are the mean+S.E. of the number of experiments indicated.

TABLE 4. Effect of varying pH_e on cellular electrolytes, pH_i and amylase release in HCO_3^-/CO_2 free incubation medium, buffered with HEPES

	Intracellular electrolytes				Amylase release (% total/30 min)			
Extracellular acid-base status	pH_i	$[K^+]_i$ (mM)	$[Na^+]_i$ (mM)	n	Control	Carbachol (10^{-6} M)	CCK_8 (7×10^{-11} M)	n
pH_e 7·4	6·77	140	55·4	4	3·3	14·2	10·3	4
	±0·06	±4·5	±3·7		±0·2	±0·3	±0·4	
pH_e 6·8	6·18	146	51·3	3	2·7	9·3	8·1	3
	±0·12	±5·7	±2·6		±0·2	±2·1	±0·9	

All values are the mean±S.E. of the number of experiments indicated.

constant at 7·4 by increasing the concentration of HCO_3^- from 25 to 100 mM. Equilibration of KHB with 20% CO_2 without changes in the HCO_3^- concentration which resulted in a pH_e of 6·80 decreased pH_i and inhibited secretagogue-induced amylase release by about 30–40%, while $[K^+]_i$ and $[Na^+]_i$ were approximately the same as under control conditions.

Effect of withdrawal of HCO_3^-/CO_2. To test whether transmembrane shifts of HCO_3^- across the acinar cell membrane could be important in the observed changes in pH_i when pH_e was altered, cells were incubated for 30 min in HCO_3^- free medium, buffered with HEPES and equilibrated with 100% O_2. As indicated in Table 4, pH_i still decreased from 6·77 to 6·18 when pH_e was reduced from 7·4 to 6·8. Secretagogue-induced amylase release was inhibited by about 25–40%, while the cellular concentrations of Na and K remained unaltered. Thus, though the pH_i of acini

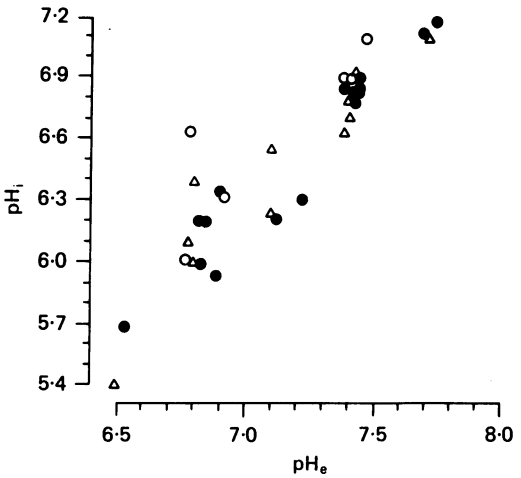

Fig. 3. Dependence of pH_i on pH_e in various incubation media in which the concentrations of CO_2 and HCO_3^- were changed independently. Summarized data of twelve experiments; each point is the mean of six determinations. Incubation media: (●) KHB equilibrated with 95% O_2/5% CO_2, HCO_3^- varied; (○) KHB equilibrated with 80% O_2/20% CO_2, HCO_3^- varied; (△) HR equilibrated with 100% O_2, pH_e adjusted with NaOH.

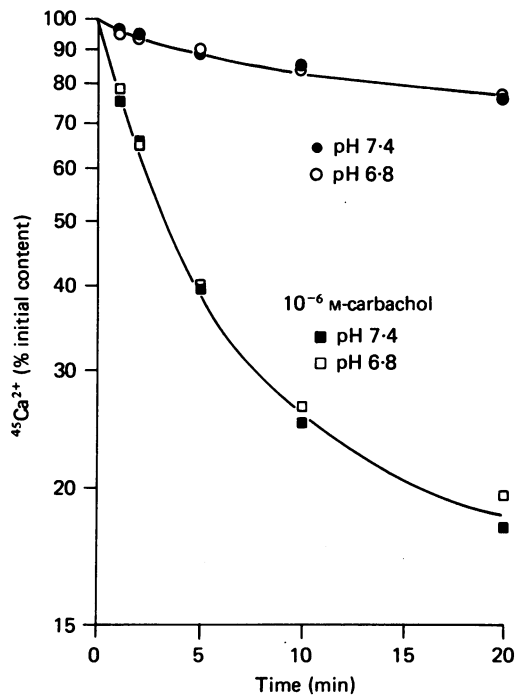

Fig. 4. Effect of varying pH_e on $^{45}Ca^{2+}$ efflux from isolated acini in the absence and in the presence of 10^{-6} M-carbachol. Acini were incubated for 1 h in KHB containing 2 μCi/ml $^{45}Ca^{2+}$ (total Ca^{2+} = 1·28 mM), then centrifuged, washed and resuspended in $^{45}Ca^{2+}$ free medium, containing the same concentration of nonradioactive Ca. $^{45}Ca^{2+}$ remaining in acinar cells at any time point is expressed as percent of total $^{45}Ca^{2+}$ present at the beginning of the washout period. Representative experiment of three.

incubated in HR (6·77) was more acid than that of acini incubated in KHB, the influence of pH_e on pH_i, cellular electrolytes and amylase release was similar in both HR and KHB. The consistency of the dependency of pH_i on pH_e at various incubation conditions is illustrated by the pooled data shown in Fig. 3.

Effect of varying pH_e on $^{45}Ca^{2+}$ efflux. Since it is believed that secretagogue-induced secretion of digestive enzymes from pancreatic acini is mediated by mobilization of intracellular Ca^{2+} (Case, 1978; Chandler, 1978; Williams, 1980), we determined whether intracellular acidification would alter $^{45}Ca^{2+}$ efflux from unstimulated and stimulated pancreatic acinar cells (Fig. 4). Neither the basal nor the increased release of $^{45}Ca^{2+}$ induced by 10^{-6} M-carbachol was altered by decreasing pH_e from 7·4 to 6·8. Thus the reduced amylase release induced by carbachol at pH 6·8 does not appear to be due to a decrease in mobilization of cellular Ca^{2+}.

DISCUSSION

Isolated pancreatic acini were used to study the effect of secretagogues on cellular electrolytes and pH because of the enrichment of acinar cells and because of the ease of controlling the concentration of markers in the extra-cellular space (Williams *et al.* 1978). Previously, pancreatic cellular electrolytes have been measured twice, once in whole rat pancreas *in vivo* (Schneyer & Schneyer, 1960) and secondly using fragments of mouse pancreas *in vitro* (Williams, 1975). In the present work, as well as the two previous studies, the intracellular potassium concentration was greater than 140 mM and the intracellular chloride concentration about 40–50 mM. The intracellular Na concentration of 50 mM determined here in acini was similar to that previously determined for mouse pancreatic fragments *in vitro* of 42 mM (Williams, 1975) but more than twice the value of 18·5 mM calculated for rat pancreatic cells in whole pancreas (Schneyer & Schneyer, 1960). It is not clear whether this discrepancy is species specific or due to the tissue preparation. The present data also indicate that the total intracellular sodium of acinar cells is exchangeable with ^{22}Na. Even though all the Na^+ is exchangeable, some may be protein bound since the sum of the intracellular Na^+ and K^+ clearly exceeds that in the extracellular medium.

Electrophysiological investigations have indicated that agents such as acetylcholine and cholecystokinin analogues which induce enzyme secretion from pancreatic acini also increase the membrane permeability to Na^+ and Cl^- (Iwatsuki & Petersen, 1977; Petersen & Philpott, 1980). This interpretation has been supported by isotope flux measurements showing that secretagogues induced an increase in the rate of $^{22}Na^+$ uptake into fragments of rat pancreas (Case *et al.* 1978; Bobinski & Kelly, 1979), and an increase in $^{22}Na^+$ as well as $^{36}Cl^-$ uptake into dispersed pancreatic acinar cells (Putney *et al.* 1980; Putney & Van de Walle, 1980).

As Na can release Ca^{2+} from mitochondria in a number of other cell types (Crompton *et al.* 1977, 1978) the possibility exists that secretagogue-induced Na^+ influx leads to a rise in the intracellular Na^+ concentration which might be the mechanism by which sequestered Ca is released into the cytoplasm (Case & Clausen, 1973; Williams, 1975). In the present study, however, carbachol failed to influence the intracellular concentrations of Na^+ as well as Cl^- and K^+, even at a concentration of 10^{-6} M which induces maximum stimulation of amylase release by these acini

(Williams *et al.* 1978). Carbachol was also without any effect on the steady state $^{22}Na^+$ uptake which excludes the possibility that there is a smaller exchangeable pool of intracellular Na^+ influenced by secretagogues not detectable by measuring total intracellular electrolytes. These results indicate that if there is an increased influx of Na into acinar cells during secretion, it is probably pumped out to maintain a steady state, possibly by the activity of a Na/K-pump, which has been shown to be present in the acinar cell membrane (Petersen, 1973; Bundgaard, Møller & Poulsen, 1979).

The possible role of sodium in the stimulus–secretion coupling of pancreatic enzyme secretion has also been pointed out by a number of investigations indicating that replacing extracellular sodium with other cations leads to an inhibition of enzyme secretion from isolated rat pancreas (Case & Clausen, 1973), mouse and rat pancreatic fragments (Williams, 1975; Petersen & Ueda, 1976) and perfused rat pancreas (Kanno, Saito & Sato, 1977). However, recent studies with isolated acini showed that Na^+ replacement failed to block amylase release and ^{45}Ca efflux (Williams, 1980). Na^+ is known, however, to be necessary for pancreatic fluid secretion (Rothman & Brooks, 1965) and blockage of secretion of pancreatic juice probably explains the earlier results where Na^+ removal inhibited enzyme secretion.

Changes in intracellular pH can affect intracellular Ca^{2+} levels in other cell types in which Ca^{2+} is important in regulation of stimulus–contraction (Carvalho & Leo, 1967; Williamson *et al.* 1975) and stimulus–secretion coupling (Malaisse *et al.* 1980). At present little is known about acinar cell intracellular pH and its relationship to the extracellular acid-base status. Taking together all our present intracellular pH measurements performed during incubation with KHB at an extracellular pH of 7·42, a mean normal intracellular pH of 6·96 could be calculated; this value is in agreement with intracellular pH values which have been determined to be 6·90–7·00 in most mammalian cells (Waddell & Bates, 1969). The H^+ equilibrium potential for acini can be calculated by use of the Nernst equation as $-28{\cdot}3$ mV. The resting membrane potential of isolated acinar cells is not known. Assuming that it is about -40 mV, however, as measured from mouse pancreatic segments (Petersen, 1976), an electrochemical gradient for H^+ ions across the membrane of about 11·7 mV is found, showing that in the resting acinar cell, H^+ is not passively distributed. This implies a net passive H^+ influx with some compensatory active efflux. Carbachol, at concentrations which maximally increase amylase secretion and Ca^{2+} mobilization in acinar cells, had no effect on intracellular pH. Thus a change in pH_i is not part of stimulus–secretion coupling in acinar cells. Interestingly, since carbachol depolarizes acinar cells to about -25 mV, H^+ will then be at equilibrium or slightly out of equilibrium with a net passive efflux of H^+. This suggests that pH_i itself is not affected by changes in the membrane potential.

In early studies on nerve and muscle cells, changes in the CO_2 tension altered intracellular pH, whereas changes in the extracellular HCO_3^- concentration at constant CO_2 were without remarkable effect; thus it was suggested that most cell membranes are freely permeable to carbon dioxide but much less permeable to bicarbonate (Caldwell, 1958; Waddell & Butler, 1959; Waddell & Bates, 1969). However, this assumption has not proved to be true for a number of other cell types including heart, kidney and lymphocytes where it was shown that intracellular pH could be changed by variation of both external HCO_3^- and CO_2 concentrations (Zieve,

Haghshenass & Krevans, 1967; Poole-Wilson & Cameron, 1975; Kleinman, Brown, Ware & Schwartz, 1980). Our data indicate that this is also true for pancreatic acinar cells. The intracellular pH changed linearly with the extracellular pH, independently of whether the extracellular pH was varied by changes in the HCO_3^- concentration at constant CO_2 or by changes in the CO_2 concentration at constant HCO_3^-. The slope of this relationship indicates a change of about 1·2–1·3 intracellular pH units for one unit change in extracellular pH. However, an increase in the extracellular CO_2 concentration failed to affect the intracellular pH, if at the same time the HCO_3^- concentration was revised to keep the extracellular pH constant. This contrasts with studies on skeletal muscle cells (Waddell & Butler, 1959; Brown, Kim & Moorhead, 1967) and liver cells (Longmore, Niethe & McDaniel, 1969) which show that the intracellular pH decreased when the CO_2 concentration was increased, independently of the extracellular pH. The linear relationship between extra and intracellular pH was also observed in HCO_3^-/CO_2 free, HEPES buffered incubation media, indicating that the change in pH_i following a change in pH_e is due to rapid equilibration of H^+ or OH^- ions, rather than to movements of HCO_3^- across the plasma membrane. Thus, pancreatic acinar cells appear to differ from many other cells in having a greater permeability to H^+ or OH^-.

In a recent study an increase in CO_2 tension was shown to lead to an electrical uncoupling of acinar cells while a comparable fall in extracellular pH produced by lowering HCO_3^- did not uncouple the cells (Iwatsuki & Petersen, 1979). These authors concluded that a fall in pH_i was responsible. Our results, however, would indicate that the effect they observed should be more directly related to CO_2 itself.

To determine whether extracellular or intracellular pH might affect cellular functions involved in stimulus–secretion coupling the pH was changed by varying the extracellular acid–base status and the effects on intracellular pH and electrolytes, amylase secretion and $^{45}Ca^{2+}$ efflux were investigated. Our initial studies showed that amylase secretion in response to both carbachol and CCK_8 was reduced when the bathing pH fell below 7·1. We were unable to determine whether this reduced secretion was due to changes in extracellular or intracellular pH because intracellular pH directly followed extracellular pH. Since the effect appears to be on an intracellular site in the stimulus-secretion coupling pathway, however, it seems likely that it is intracellular acidity that is altering amylase release.

Operationally we can divide pancreatic stimulus–secretion coupling into the sequential steps of receptor occupancy, Ca^{2+} mobilization and the action of Ca^{2+} to promote exocytosis. In the present study the response to agonists acting on both the cholinergic and the CCK receptor was comparably inhibited by acid pH. We have recently characterized the pH optimum of the CCK receptor on mouse acini and found increased binding of radioiodinated CCK at acid pH (H. Sankaran & J. A. Williams, unpublished data). It thus seems unlikely that the decreased amylase release seen with an acid pH is due to an effect at the receptor level. More to the point, $^{45}Ca^{2+}$ mobilization, if anything, is slightly enhanced when acini were studied at pH 6·8 as compared to 7·4. This indicates that neither receptor occupancy or Ca^{2+} mobilization are being affected, but rather, that some latter step by which Ca^{2+} activates exocytosis is reduced at acid pH. This could involve a Ca^{2+} receptor such as calmodulin or changes in Ca^{2+} activated kinase enzymes or substrates.

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